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## A Large Animal Model of Burn Hypermetabolism<sup>1</sup>

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Further research on postburn hypermetabolism is limited by the constraints of patient studies and the reduced responses of small animal models. To test the validity of a large animal model, oxygen uptake ( $\dot{V}_{O_2}$ ), urinary excretion of epinephrine (E), norepinephrine (NE), and dopamine (DA), and rectal temperature were monitored in conscious 20- to 40-kg goats for 3 weeks following a 25% total body surface burn. While  $\dot{V}_{O_2}$  and catecholamine output remained at control levels in sham burned animals,  $\dot{V}_{O_2}$  rose from  $5.57 \pm 0.23$  to  $6.66 \pm 0.31$  ml/min·kg (mean  $\pm$  SEM) in one group of seven injured animals 8 to 10 days postinjury and from  $4.58 \pm 0.17$  to  $6.30 \pm 0.07$  in another group ( $n = 6$ ) studied 19 to 21 days postinjury. Excretion of E and NE in four injured goats was three to four times that in four sham-burned animals 7, 14, and 21 days postburn. Dopamine output was comparable in the two groups. There was no measurable change in rectal temperature after injury. Injured animals maintained body weight and did not become bacteremic. The hypermetabolic and neuroendocrine responses of the injured goat make this large animal an appropriate model for further research.

### INTRODUCTION

The hypermetabolic response to injury was first described in patients with long bone fractures in 1930 [4]. Since that time, numerous investigators have confirmed and extended these initial observations to demonstrate that the increase in metabolic rate is (a) common in a wide variety of surgical patients, (b) related to the extent of injury, and (c) greatest following severe burns [18, 19].

<sup>1</sup> The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. In conducting the research described in this report the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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Many basic components of postinjury hypermetabolism, however, continue to elude clinical investigators due to the constraints of human research. Attempts to develop small animal models have been hampered by both the animals' size and their relatively limited response to injury [2, 5, 13]. Sheep [20] and dogs [17] have been utilized to study the acute cardiovascular adjustments to burn injury, but the associated metabolic responses of these animals have not been recorded. The purpose of this study is to determine whether thermal injury causes a reproducible and, therefore, predictable increase in resting oxygen consumption of the goat. Because hypermetabolic burn patients are febrile and excrete increased quantities of epinephrine and norepinephrine, additional efforts to validate the goat model include measurements of core temperature and urinary excretion of catecholamines.

### MATERIALS AND METHODS

Young, healthy, castrated male and non-pregnant female goats of mixed breeds were

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utilized as experimental animals. Upon arrival, they were given anthelmintics and housed individually in outdoor runs for at least 2 weeks. Animals weighed between 20 and 40 kg, were fed Wayne Ruff 'N Redi 12 Complete Horse Feed and alfalfa hay, and given water *ad libitum*.

After the initial period of adjustment, goats were moved into the laboratory where room temperature was maintained between 25 and 28°C. Over the next 3 weeks, the animals were conditioned to stand quietly for 1 hr in a small stand. The goat stood in a nylon mesh sling to prevent the animal from stepping off the stand, and the horns were tethered to an overhead bar to limit head movement. At the end of each training session, rectal temperature was taken with a standard glass thermometer, and the animal was weighed on a platform balance.

Once conditioned, each animal was placed in one of two basic protocols. In one, oxygen consumption was measured before and after receiving a 25% total body surface burn. In the other, urinary catecholamine excretion was determined before and after the same injury. Uninjured animals served as controls in both protocols. Environmental conditions and method of confinement were the same for all studies.

*Oxygen consumption* was determined in tracheostomized goats by closed circuit spirometry [3]. Measurements were performed once or twice daily for 3 days beginning the day after tracheostomy. On the day of study, a disposable, cuffed, tracheostomy tube was inserted, the cuff inflated, and the animal left undisturbed on the stand for 30 to 45 min prior to spirometry (Fig. 1). The tracheostomy tube was then attached to a two-way, low-resistance Rudolph valve which was in turn connected to a calibrated, 9-liter, Collins spirometer (W. E. Collins, Inc. Braintree, Mass.) by large-bore respiratory tubing. The valve permitted unidirectional air flow between the goat and the spirometer so that the animal inhaled 100% oxygen from the spirometer and exhaled through a separate line into a carbon dioxide absorber

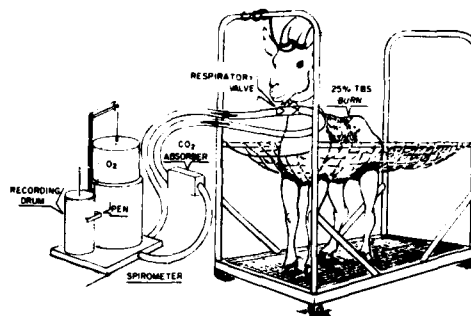


FIG. 1. Closed circuit spirometry in goat model.

and back into the spirometer. The rate of decrease in oxygen volume was recorded for 15 to 20 min and this slope used to calculate the animal's oxygen uptake. All gas volumes were corrected to standard conditions and oxygen consumption was expressed in milliliters O<sub>2</sub> per minute per kilogram body weight.

The tracheostomy tube was removed immediately after each study and the animal's rectal temperature and body weight were recorded. The frequency of testing depended on the general activity level of the animal. Most stood quietly and were studied once daily. Two animals had to be dropped from the study due to their inability to accept the experimental setup and stand quietly.

Following control studies, anesthesia was induced in 13 animals by intravenous methohexital sodium (10 to 15 mg/kg) and maintained at a surgical plane with a mixture of methoxyfluorane and 100% oxygen. Hair was clipped from the back and both sides and a third degree flame burn created over 20 to 25% of the total body surface.<sup>4</sup> Six goats were anesthetized and their hair was clipped as described, but these goats were not injured. They were designated the "sham-

<sup>4</sup> The area to be burned was carefully circumscribed and then traced onto a clear plastic overlay. Measurement of this overlay provided an estimate of the surface area of the burn. Total body surface area was calculated by the standard formula: surface area (m<sup>2</sup>) = K \* weight<sup>2/3</sup>. Since the precise K for goats is unknown, that for sheared sheep (0.084) was used [6]. Burn depth was established histologically.

burned" animals and treated in exactly the same manner as the injured animals over the course of study.

The goats were allowed to recover spontaneously without fluid or electrolyte administration. For the remainder of the study, all animals received a daily supplement of 1000 cal (Ensure, Ross Laboratories, Columbus, Ohio) by gavage in addition to the regular diet. The daily conditioning program continued as before. In one group of seven injured animals (Burn Group I) and six sham-burned animals (Sham Burn Group I), a second tracheostomy was performed distal to the previous site on the 7th day postinjury. Oxygen consumption measurements were repeated in these two groups on the 8th, 9th, and 10th days postinjury. In another group of six injured goats (Burn Group II), the second tracheostomy was performed on the 18th day postburn and spirometry conducted over the next 3 days.

No systemic or topical antibiotic therapy was used. Venous blood samples were obtained the last 2 days of study and bacteriological cultures were performed. Animals were euthanized at the end of the study, necropsies performed, and wound biopsies obtained for histological examination.

*Urinary excretion of catecholamines* was determined in eight goats following the same general protocol. Four injured animals made up Burn Group III, and four sham-burned animals were designated Sham Burn Group II. Four control studies were performed on each animal, and single studies were repeated 7, 14, and 21 days postinjury or sham burn. To avoid the stress of urethral catheterization, a catch-pan was constructed for the goat stand and urine was collected when the animal voided normally. Urine samples were acidified immediately upon collection, and epinephrine, norepinephrine, and dopamine contents determined by reverse-phase, high-pressure liquid chromatography with electrochemical detection [19].

Prior to the control runs, each goat was given a liter of water by gavage to promote urine production. On subsequent studies, 1

liter of the dietary supplement was used instead of water. Depending on the initial level of hydration and resultant urinary frequency, the collection periods ranged from 3 to 14 hr. While the animal may have voided more than once during this time, excretion rates were determined as the average for the entire period and expressed in nanograms per minute per kilogram of body weight.

All data are presented as group mean  $\pm$  SEM. Student's *t* test for paired data was used to evaluate changes in oxygen consumption following injury or sham burn. All studies were included in this analysis. The average oxygen consumption of each goat before injury was compared with the average response of that same animal after injury or sham burn. An analysis of variance was performed on the catecholamine data.

## RESULTS

The 25% total body surface burn was well-tolerated by all animals. During the first 1 or 2 days of recovery, injured goats drank liberal quantities of water but appetite was usually depressed. By the third or fourth day postinjury, they began eating normally and, in general, maintained body weight over the period of observation (Table 1). No animal appeared to develop a significant bacteremia as judged by the absence of positive blood stream cultures and clinical symptoms (i.e., fever, anorexia, coughing, nasal discharge, etc.). In general, the wound did not begin to separate until late in the second week postinjury, and there was never any histological evidence of burn wound invasion. Three goats had to be excluded from study due to pulmonary complications<sup>5</sup> rather than any direct result of the burn itself.

*Oxygen consumption* ( $\dot{V}_{O_2}$ ) remained at control levels in the sham-burned animals but was increased in the two groups of injured goats (Table 2). The average increase

<sup>5</sup> Two uninjured goats developed excessive soft tissue swelling around the tracheostomy site; the third developed a lung abscess.

TABLE 1  
EFFECT OF THERMAL INJURY ON BODY WEIGHT<sup>a</sup>

Group	Days postinjury			
	-5 to 0	7 to 10	13 to 15	19 to 21
Sham burn I	26.4 ± 2.1	26.2 ± 2.2	—	—
Burn I	28.3 ± 1.0	28.6 ± 1.0	—	—
Sham burn II	33.1 ± 0.6	33.6 ± 1.3	34.5 ± 1.9	35.4 ± 1.6
Burn II	29.6 ± 1.2	29.2 ± 0.5	29.0 ± 0.5	28.0 ± 0.9
Burn III	35.3 ± 1.0	33.9 ± 2.2	33.8 ± 2.2	34.6 ± 2.4

<sup>a</sup> Body weight in kilograms; mean ± SEM; weight changes were nonsignificant.

in  $\dot{V}_{O_2}$  was 19.6% above control levels in the group of seven animals studied 8 to 10 days postinjury (Burn Group I) and 37.6% in the six goats of Burn Group II retested 3 weeks postburn. This difference in the percentage increase in  $\dot{V}_{O_2}$  following injury developed as a result of lower control values in Burn Group II rather than any absolute difference in postburn  $\dot{V}_{O_2}$  of the two groups.

Over the three-week period of observation, catecholamine excretion remained at control levels in the sham-burned animals, but in the injured goats, epinephrine and norepinephrine outputs rose above preinjury values by the seventh day postinjury and remained elevated 14 and 21 days postburn (Table 3). The elevated rates of catecholamine excretion were reasonably stable over this period of time. While all injured animals maintained increased norepinephrine excretion

rates, one of the four failed to elevate epinephrine output above the control range (0.17, 0.07, and 0.16 ng/min·kg at 7, 14, and 21 days postinjury). Therefore, while excretion of both amines was significantly elevated in the injured group, thermal injury had a more consistent effect on norepinephrine output. Dopamine excretion was highly variable and not significantly elevated at any point following injury.

Thermal injury did not result in a significant change in rectal temperature over the 3-week period (Table 4). On occasion, the injured animals appeared to shiver, but such behavior was associated with handling (i.e., tube feeding, weighing, etc.) and would disappear as soon as the animal was left alone.

## DISCUSSION

Average oxygen consumption of all uninjured animals was within the normal range for goats studied under comparable experimental conditions [12, 16]. Therefore, the observed difference in control values between the two burn groups (Table 2) was considered a function of normal variation.

Injured animals became hypermetabolic as early as 8 days postinjury and remained so for the next 2 weeks. The 20% increase in  $\dot{V}_{O_2}$  of animals studied 8 to 10 days postinjury was about half that observed in the group at 3 weeks postburn. Since this difference was the result of lower control level in the latter group, rather than any differ-

TABLE 2  
EFFECT OF THERMAL INJURY ON OXYGEN CONSUMPTION

Group	Days postinjury		
	-3 to 0	8 to 10	19 to 21
Sham			
burn I	5.49 ± 0.45	5.54 ± 0.44	—
Burn I	5.57 ± 0.23	6.66 ± 0.31*	—
Burn II	4.58 ± 0.17	—	6.30 ± 0.07**

Note. Oxygen consumption in milliliters  $O_2$  per minute per kilogram body weight; mean ± SEM. Paired *t* test compared with same group of animals before injury; \* *P* < 0.05, \*\* *P* < 0.01.

TABLE 3  
EFFECT OF THERMAL INJURY ON URINARY CATECHOLAMINE EXCRETION

	Days postinjury			
	0	7	14	21
Norepinephrine				
Sham burn II	0.34 ± 0.03	0.36 ± 0.05	0.28 ± 0.06	0.23 ± 0.04
Burn III	0.28 ± 0.03	1.03 ± 0.08*	0.78 ± 0.18*	0.89 ± 0.26*
Epinephrine				
Sham burn II	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.04	0.13 ± 0.02
Burn III	0.18 ± 0.04	0.53 ± 0.14**	0.35 ± 0.08**	0.41 ± 0.18**
Dopamine				
Sham burn II	1.32 ± 0.18	1.28 ± 0.20	1.34 ± 0.20	0.93 ± 0.24
Burn III	1.23 ± 0.20	2.36 ± 0.40	1.91 ± 0.38	2.45 ± 0.84

Note. Excretion rate in nanograms per minute per kilogram body weight; mean ± SEM. Analysis of variance comparing burn and sham-burned animals at 7, 14, and 21 days; \*  $P < 0.01$ , \*\*  $P < 0.05$ .

ence in  $\dot{V}_{O_2}$  at 1 and 3 weeks postinjury, it is doubtful that the metabolism of the injured animal continued to increase for 3 weeks. Alternatively, the lower control  $\dot{V}_{O_2}$  values may more accurately represent resting aerobic metabolism and thereby make the 40% increase in  $\dot{V}_{O_2}$  of this group a better estimate of the actual energy cost of injury. In addition, the general activity level of these animals decreased slightly following injury, so whether the 20 or 40% figure is chosen, it should be considered a minimal estimate of actual postburn hypermetabolism. Even if the 40% estimate is selected, it is only about half that anticipated in a burned human with the same size injury [22].

Herndon *et al.* [14] found about the same increase in  $\dot{V}_{O_2}$  of rats and guinea pigs following 50% total body surface burns. Rats with smaller injuries (burns of a size comparable to that in the goat model) have a more limited metabolic response and most, if not all, of this extra metabolism can be eliminated by increasing ambient temperature [3, 7, 14]. That the energy cost of thermal injury appears to vary with body size (from rat to man) may eventually provide some insight into the basis of burn hypermetabolism.

Under these experimental conditions, the injured goat failed to demonstrate a measureable increase in rectal temperature (Ta-

TABLE 4  
EFFECT OF THERMAL INJURY ON RECTAL TEMPERATURE\*

Group	Days postinjury			
	-5 to 0	7 to 10	13 to 15	19 to 21
Sham burn I	39.5 ± 0.2	39.8 ± 0.2	—	—
Burn I	39.4 ± 0.1	39.5 ± 0.2	—	—
Sham burn II	39.0 ± 0.1	39.1 ± 0.04	39.0 ± 0.05	39.0 ± 0.05
Burn II	39.6 ± 0.1	39.5 ± 0.04	39.6 ± 0.2	39.8 ± 0.1
Burn III	38.8 ± 0.1	39.2 ± 0.2	39.2 ± 0.1	39.3 ± 0.2

\* Expressed in °C; mean ± SEM.

ble 4). Considering the variability in control values observed in this and other studies [1, 13, 15] subtle changes in core temperatures following injury could easily go unnoticed. Consequently, before a more definitive statement can be made regarding the thermoregulatory response of this animal, changes in experimental design are necessary. A large animal environmental chamber is currently under development at this laboratory to address this issue.

All animals in this study were housed in an ambient environment well within the thermoneutral zone for an uninjured goat [2], and injured animals did not appear to be cold. But, since goats may develop a degree of nonshivering thermogenesis [1], which triggers metabolic and neuroendocrine responses comparable to those observed in the injured animals, the thermoregulatory contribution to postburn hypermetabolism must be clarified in this particular model.

Norepinephrine excretion of the uninjured animals was comparable to that reported in other normal control goats [8]. Epinephrine output, on the other hand, was twice the reported normal value. Similar shifts in the epinephrine-to-norepinephrine ratio have been observed in baboons and rhesus monkeys studied in primate chairs and are considered a function of confinement [9].

The three- to fourfold increase in urinary excretion of epinephrine and norepinephrine of the injured animals was of the order of magnitude reported in four patients with comparable size burns and studied at the same time postinjury [11]. The failure of one animal to increase epinephrine output was unexplained but suggests that factors other than the burn itself may influence postinjury adrenal medullary activity in the goat.

In the injured goat model, dopamine excretion was highly variable and not consistently above control levels at any time postinjury. Dopamine turnover is markedly accelerated in the burn patient where a major portion is rapidly incorporated into norepinephrine [10]. Presumably, the rapid rate

of dopamine turnover made it impossible to demonstrate a rise in urinary excretion in the injured goat.

The relationship between increased catecholamine excretion and oxygen consumption was described in burn patients as early as 1967 by Harrison *et al.* [12]. Since the calorogenic potential of catecholamines had already been established, they concluded that increased sympathoadrenal activity was responsible for the elevation in metabolic rate of burned patients. This hypothesis was later confirmed by Wilmore and collaborators [22] by first demonstrating a significant relationship between metabolic rate and urinary catecholamine excretion and then reducing the hypermetabolism by adrenergic blockade. The results of this study, while obtained in different groups of animals, indicate that the increase in aerobic metabolism of the goat was associated with a simultaneous increase in sympathoadrenal activity. The direct cause and effect relationship must be established in the goat, but the combined neuroendocrine and metabolic responses of this animal to thermal injury strongly suggest that it is a suitable model for further research.

## SUMMARY

A 25% total body surface burn increased oxygen consumption of the goat by 20 to 40%. This hypermetabolism was apparent as early as 8 to 10 days postinjury and remained evident at 19 to 21 days postburn. Associated with these changes in aerobic metabolism was a three- to fourfold increase in urinary epinephrine and norepinephrine excretion which again was evident 1 week postinjury and persisted for at least 3 weeks following injury. Dopamine excretion was highly variable but not significantly affected by injury. While the thermoregulatory adjustment of this large animal model remains uncertain, changes in oxygen consumption and urinary catecholamine excretion in the goat are very much like the human response to thermal injury.

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